Receptor Mechanisms and Dose–Response Models for the Effects of Dioxins

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There is increasing evidence that receptor-mediated events impact one or more stages responsible for tumor development in experimental animals and humans. Although many chemicals and endogenous hormones require receptor interactions as a necessary event in their carcinogenic activity, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and its structural analogs are the most visible examples of receptor-mediated carcinogens. TCDD, or dioxin as it is frequently called, interacts with the Ah receptor (AhR), which functions in a manner analogous to receptors for steroids. TCDD produces a wide spectrum of biochemical and toxic responses in *in vitro* and *in vivo* systems, and the Ah receptor is generally considered necessary for most if not all of these responses. Risk assessments for dioxin made by the United States and other countries throughout the world have been based on its carcinogenecity in experimental animals. Recently, epidemiology studies have indicated that TCDD is a human carcinogen at high doses. Because TCDD appears to be acting like a potent and persistent hormone agonist, it appears reasonable to incorporate mechanistic information on receptor-mediated events in risk assessments for TCDD. This information may be obtained from steroid receptor action and from molecular data on the Ah receptor. In this paper, we evaluate the scientific foundation on which mechanistic models for estimating dioxin's risks should be based. These models need to recognize the mechanisms possible for the diversity of biological responses that are initiated by a single receptor interacting with a single ligand. The U.S. EPA is currently reevaluating dioxin's risks by examining the possibility of developing biologically based models. This paper details the considerations that must be made in developing such models, including information on mechanisms of steroid hormone action, characteristics of the Ah receptor, diversity of receptor actions, and design issues that are crucial for the translation of biological phenomenon into valid dose–response models. *Environ Health Perspect 101(1):36–44*

Analysis of carcinogenicity and genetic toxicity data for chemicals subjected to the National Toxicology Program (NTP) bioassay for carcinogenicity have demonstrated that nearly 40% of the chemicals that were positive in the bioassay were negative in tests for genetic toxicity (*1*). These findings, together with evidence of the role of cell proliferation in chemical carcinogenesis, provide further evidence that receptor-mediated responses may play a critical role in the carcinogenic actions of many chemicals (*2,3*). Of particular interest are those receptor-mediated events that trigger mitogenic responses in normal and genetically altered cells.

There is increasing controversy regarding risk assessment procedures for chemical carcinogens. At the forefront of this controversy is the issue of the appropriateness of linear models or safety factor approaches for estimating human risks. The linear multistage model is commonly used by regulatory agencies, and it assumes that the risk is proportionately related to dose/exposure level in the low-dose region of the dose–response curve. In contrast, safety factor models assume that there is an exposure level below which no adverse effect can occur. There is little disagreement over the use of the linear multistage model for carcinogens that appear to act by damaging DNA in such a way that somatic mutations are produced. The assumption is that one genetically altered cell can eventually lead to cancer, although it is now clearly recognized that cancer is a multistage pro-cess involving several mutations and multiple mechanisms governing selective growth of these genetically altered cells (*2,4*).

The risk assessment controversy surrounds policies for assessing risks of the so-called nongenotoxic carcinogens: those substances that do not produce somatic mutations but instead increase the growth rates of normal and/or abnormal cells, thereby increasing risks of tumor development. One opinion is that this class of carcinogens should exhibit a threshold and that a safety factor approach is the most appropriate for estimating human risks. Others argue that the group of chemicals that make up the class of non-genotoxic carcinogens act by several different mechanisms, and it is misleading and scientifically incorrect to treat all nongenotoxic chemicals the same for risk assessment purposes. For example, one chemical might stimulate mitotic activity by modifying receptor-mediated signal transduction pathways, whereas another chemical might also stimulate cell division but by a compensatory mechanism linked to cytolethality (*5*). It is likely that dose–response relationships for these two mechanisms will be very different.

There has been considerable debate over the relevance of using high-dose animal data to estimate human risks caused by chronic low-dose exposure. Several reports have been published on this issue, so we will not address it here (*6–8*). Instead, we focus on the emerging insights regarding dose–response relationships for receptor-mediated events and the application of this information to developing novel mathematical models that provide the foundation to use receptor mechanisms in risk assessment.

Although the carcinogenic actions of many chemicals such as estrogens and phorbol esters are mediated through receptor mechanisms, the most visible example of a receptor-mediated carcinogen is dioxin. Risk assessments for dioxin and its structural analogs have created an unprecedented emotional and economic controversy which include the issues of health effects in Vietnam veterans, fraud, collusion, and overregulation (*9,10*). EPA is currently reevaluating

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health risks associated with dioxin exposure, and this decision has spawned charges of collusion between federal officials and industry. Here we evaluate the strength of the scientific foundation on which the EPA reevaluation is based, including an examination of mechanisms of receptor actions and the scientific issues that affect the development and use of biologically based risk assessment models.

Biochemical and Toxic Effects of Dioxins

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) symbolizes the intense controversy surrounding the issue of dose–response relationships in receptor-mediated carcinogens. TCDD is loosely referred to as "dioxin." Dioxin is prototypical of a large class of organohalogens including several polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), a few polychlorinated biphenyls (PCBs), and other less common contaminants [see review by Goldstein and Safe (*11*)]. The PCDDs and PCDFs are ubiquitous environmental contaminants that arise inadvertently during the synthesis of other compounds such as the herbicide 2,4,5-trichlorophenoxyacetic acid, the active ingredient in Agent Orange, and in other processes such as bleaching paper and pulp and incinerating hazardous wastes. Many of the PCDFs and PCDDs are resistant to degradation in the environment and in biological systems. For example, the concentrations of these compounds are biomagnified in aquatic systems, and the whole-body half-life in humans for TCDD is in the range of 7–11 years (*12*). Studies in the early 1970s revealed that TCDD possesses extraordinary acute toxicity in experimental animals. For example, the LD_{50} is approximately 1 μg/kg in guinea pigs and 70 μg/kg in rats (*13*). Later studies demonstrated that TCDD is a multisite carcinogen in several experimental animal models, and it is a carcinogen in both sexes, in some cases at doses well below the maximum tolerated dose (*14,15*). Several studies in the scientific literature have evaluated the genetic toxicology of TCDD. These studies have been reviewed by Shu et al. (*16*), and taken together they indicate that TCDD is negative in short-term tests for genetic toxicity and does not appear to form DNA adducts. However, the possibility remains that TCDD may damage DNA by indirect mechanisms such as enhanced production of DNA-reactive metabolites of other chemicals and/or endogenous compounds such as estrogens. A recent report (*17*) indicated that TCDD induced neoplastic transformation in immortalized human keratinocytes. In two-stage models for liver and skin cancer, TCDD is a potent tumor promoter with little or no initiating activity (*18–20*). However, it must be kept in mind that promotion is an operational term, and it does not convey specific information about mechanism of action. The knowledge that chemicals of diverse structure are tumor promoters and the identification of some of the discrete steps involved in tumor promotion provide growing evidence that there are multiple mechanisms responsible for tumor promotion (*4*). Reasonable evidence is now available that dioxin is a human carcinogen at least at high doses (*21*).

In addition to TCDD's carcinogenic actions, it has a number of other toxic effects such as immunotoxicity, reproductive deficits, teratogenicity, and endocrine toxicity (*22*). There is an emerging consensus that noncancer endpoints need to be considered in a complete evaluation of dioxin's effects. This consensus is based on numerous experimental findings including the growing evidence that dioxin is a powerful growth dysregulator. We agree with this consensus and recommend the development of biologically based models for all the potential health effects of dioxin.

Dioxin modulates the activities of a vast array of biochemical pathways including receptors (estrogen, glucocorticoid, epidermal growth factor), hormones, components of intermediary metabolism, transforming growth factor, tumor necrosis factor, metabolic activation/deactivation mechanisms (cytochrome P450 and uridine diphosphate glucuronyltransferase isozymes), inflammation factors, interleukins, and proto-oncogene expression (*22–24*). Our burgeoning knowledge on the wide spectrum of growth factors and growth factor pathways that are modulated or regulated by dioxin, coupled with recent information on the capacity of dioxin to enhance cell proliferation rates, may lead to the identification of critical target genes for dioxin's effects. However, the ability of dioxin to elicit toxic effects most surely requires the interaction of multiple genes. Further complexity is added by cell specificity and hormonal regulation of dioxin's effects. For example, TCDD is a potent promoter of liver tumors in intact female rats, and it also induces significant increases in mitotic activity of hepatocytes in fe-male rats (*19*). However, in ovariectom-ized rats, TCDD does not induce hepatocyte proliferation nor liver tumors, but it does promote lung tumors which are not observed in intact female rats (*25*). Clearly, estrogens play a dominant role in regulating the site specificity of the tumorgenicity of dioxin.

Ah Receptor: Lessons from Steroid Receptors

Although substantial gaps in our knowledge remain, it is generally agreed that an early event in most, if not all, of dioxin's effects require interaction with a cellular protein, the Ah receptor (AhR), which was discovered by Alan Poland in 1976 (*26*). The binding of TCDD to AhR is similar, although not identical, to the interaction of many steroid hormones with their intracellular receptors. However, steroid hormones do not bind the Ah receptor, and TCDD does not bind to known steroid receptors. Moreover, any chemical that binds to the Ah receptor appears to produce the same spectrum of biochemical and toxic effects as dioxin, and the potency of these responses reflects the binding affinity of that chemical to the Ah receptor (*11*). TCDD binds the Ah receptor with high affinity (K_d approximately 10^{-10} M), and the binding is reversible (*23,26,27*). It is generally accepted that Ah receptor occupancy is linearly related to low cellular concentrations of dioxin.

There is considerable information available on the interaction of the TCDD receptor complex with the cytochrome P4501A1 gene, which is transcriptionally activated by TCDD and its structural analogs. Cytochrome P450 is composed of numerous isozymes, and it functions in the detoxication and/or metabolic activation of many chemicals. TCDD induces the synthesis and catalytic activity of *CYP1A1* and *CYP1A2*. Much of this work is summarized in a recent review (*27*). Biochemical studies indicate that the functional form (liganded) of the receptor is a heteromer (*28*), and the functional form of the receptor appears to contain a ligand-binding domain and a DNA-binding domain. Recently, cDNA cloning approaches have shown that the ligand-binding component has a basic helix-loop-helix (bHLH) motif similar to that for some other transcription factors (*29,30*). Moreover, the Arnt (Ah receptor nuclear translocation) protein which activates the DNA binding also contains a bHLH motif (*31*). It is thought that the common bHLH motifs allow the Ah receptor and the Arnt protein to dimerize, forming the functional DNA binding complex similar to other bHLH transcription factors. Induction of transcription of the *CYP1A1* gene requires binding of the liganded receptor to specific elements in the gene, which have been termed "dioxin-responsive elements" or "xenobiotic-responsive elements" (DRE, XRE). There are multiple binding sites, and the binding is reversible. Each receptor binding site contains a core recognition sequence, and the liganded receptor appears to bind within the major DNA groove similar to other

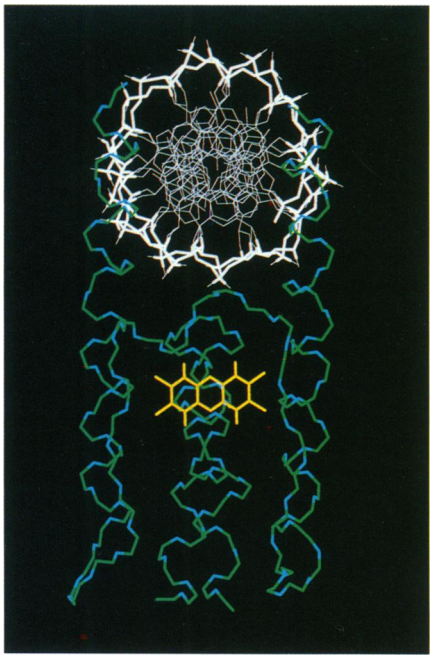


Figure 1. One possible computer-generated model of the dioxin: Ah receptor: Arnt protein: DNA complex based on the prior model (*48*) of the b–HLH (basic region, helix-loop-helix) binding to DNA (AAACATATGTTT). Backbone DNA (black), dioxin (red), and the HLH protein models of the Ah receptor and Arnt protein (blue-green). (We thank Zelda Wasserman and William Degrado for sending us their model coordinates.)

systems that are regulated by bHLH transcription factors (*27,32*). It is important to remember that the molecular characteristics that regulate induction of *CYP1A1* may be different for other dioxin-responsive genes. Figure 1 illustrates DNA interactions for bHLH transcription factors such as the liganded AhR.

Much of the sequence of molecular events governing transcriptional activation of the *CYP1A1* gene are analogous to receptor-mediated events for steroid hormones. This similarity helps us in proposing biological models of TCDD action for risk assessment purposes. The steroid hormones and their receptors belong to a multigene family that includes the thyroid hormone receptors, oncogene products, glucocorticoids, mineralocorticoids, vitamin D, retinoids, androgens, estrogens, and progestins. Biologically, these are all multipotent agents that induce a range of cellular responses in different organs, many at extremely low concentrations. Within the family of known receptors for these agents, there is considerable sequence homology and a common basic structure, consisting of a ligand-binding domain and a DNA-binding domain, as discussed above for AhR. Some receptors are associated with so-called heat shock proteins or proteins that must be shed to transform the liganded receptor into a DNA-binding form (*33*).

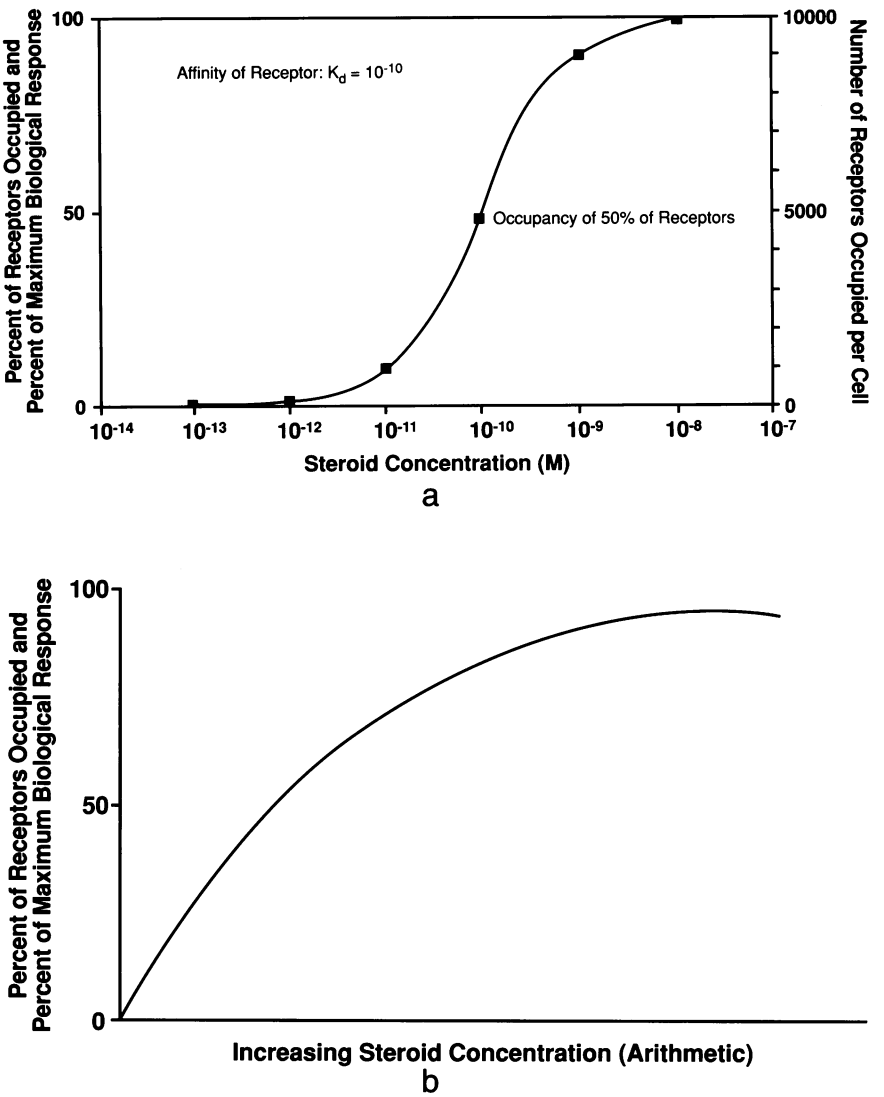


Figure 2. (a) Concentration-dependent hormone response when there is a proportional relationship among hormone concentration, receptor occupancy, and biologic response. Data are plotted on a semilog scale and demonstrate that the entire dose response spans at least six orders of magnitude. (b) Proportional relationship among hormone concentration, receptor occupancy, and biologic response using the same data set plotted in panel (a) plotted on an arithmetic scale. In this case, linearity of response is clearly seen in the low concentration region, followed by saturation at the higher concentrations.

Dose–Response Relationships for Receptor-mediated Events

Attempts to mathematically model the steps involved in signal transduction have examined events step by step as well as the overall set of reactions from entrance of hormone into the cell to cellular response. Of interest here is the information available concerning the overall dose–response relationship for hormones.

Evaluation of dose–response relationships for receptor-mediated events require information on the quantitative relationships among ligand concentration, receptor occupancy, and biologic response. According to Roth and Grunfeld (*34*):

> At very low concentrations of hormone ([H]<<K_d), receptor occupancy occurs but may be trivial; i.e., the curve approaches 0% occupancy of receptors. But if there are 10,000 receptors per cell (a reasonable number for most systems), the absolute number of complexes formed is respectable even at low hormone concentrations.

Figure 2a illustrates the situation described above where there is a proportional relationship between receptor occupancy and biological response. In this situation occupancy of one receptor would produce a response, although it is unlikely that this response could be detected. The biological significance of such a response is likely negligible, but this is not certain, and it may vary with endpoint as well as with developmental stage and cell type. Note that the data in Figure 2a are plotted on a semilog scale. If the same data are plotted

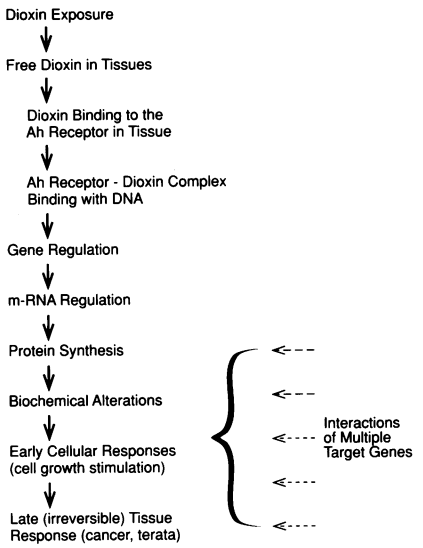


Figure 3. Schematic representation of the complex sequence of molecular and biological events involved in dioxin–mediated toxicants.

arithmetically (Fig. 2b), the shape of the dose–response curve readily conveys a linear relationship between receptor occupancy and biological response at lower concentrations and saturation at higher concentrations.

Such a simple proportional relationship does not explain the diversity of biological responses that can be elicited by a single hormone using a single receptor. For example, low concentrations of insulin produce much greater effects on fat cells than on muscle cells. These differences are due to tissue- and cell-specific factors that modulate the qualitative relationship between receptor occupancy and response. Similarly, it is expected that there are markedly different dose–response relationships for different effects of TCDD. Coordinated biological responses, such as TCDD-mediated increases in cell proliferation, likely involve other hormone systems, which means that the dose–response relationships for effects involving single genes (i.e., *CYP1A1* induction) may not accurately predict dose–response relationships for complex responses such as cancer. Indeed, recent studies have demonstrated that there are a wide variety of dose–response relationships for dioxin's effects (*3,35,36*). For example, induction of *CYP1A1* and effects on the epidermal growth factor receptor, within the framework of a two-stage model for dioxin's hepatocarcinogenic actions, indicate there is an approximate linear relationship between target tissue concentration and response even in the low-dose region. Curve-fitting approaches (*36*) as well as mechanistic models for dioxin-mediated changes in gene expression (*37,38*) provide more evidence for the idea that linear models for responses in the low-dose region cannot be rejected simply on the knowledge that a response is receptor mediated.

In contrast to dioxin's effects on *CYP1A1* and *CYP1A2*, changes in hepatocyte proliferation rates, also within the framework of a two-stage tumor promotion model, reveal that this endpoint is less sensitive to dioxin than simple changes in gene expression (*25*, R. Maronpot, personal communication). Likewise, TCDD-mediated growth of foci of cellular alteration (preneoplastic lesions) appears to be less sensitive than enzyme induction. In contrast, neoplastic transformation of human keratinocytes occurs at lower concentrations than needed to produce detectable *CYP1A1* induction (*17*). These kinds of data provide striking examples that our ability to predict dose–response relationships for the effects of dioxin are limited by our lack of knowledge on the mechanistic link between changes in gene expression and toxic effects. Figure 3 summarizes the series of interconnected steps within the three major components of receptor-mediated events (recognition, transduction, and response). Although this scheme is simplified (i.e., each step may be composed of several events), it does provide a framework for identifying knowledge gaps that create uncertainty. Clearly, interactions with other endocrine systems are involved in some effects, and our ability to construct accurate dose–response models for cancer and noncancer endpoints would be enhanced if we had a better understanding of TCDD–endocrine interactions.

In considering possible dose–response relationships for the effects of dioxin, we are hampered by our lack of knowledge concerning the absence or presence of endogenous ligand(s). We are not certain if TCDD is more or less stable than this possible ligand, or if TCDD's affinity is higher or lower than an endogenous ligand. Of course, differences in affinity, if these exist, may not influence the overall kinetics of the dose–response relationship as much as differences in the number of events required to trigger the reaction from step to step.

One of the more active areas of research on hormone action is directed at identifying the cell-specific factors that produce diversity of responses for receptor-mediated responses; that is, how do a single receptor and a single ligand produce the wide spectrum of cell-specific responses characteristic of exposure to a given hormone? Because TCDD is acting like a potent and persistent hormone agonist/antagonist, the mechanisms responsible for qualitative and quantitative differences in dose–response relationships for Ah receptor-mediated events might be similar to those mechanisms identified for steroid hormones. Fuller (*39*) has summarized some of the mechanisms responsible for generating diversity, and these are listed in Table 1.

Cancer Mechanisms and Risk Assessment

Cancer is a complex, multistage disease. Although multiple somatic mutations and multiple rounds of cell replication are involved, the sequence of those events are far from clear (*3,4*). In experimental systems, initiation is generally thought to be a DNA-damaging or altering event, and fixation is the immortalization of the mutation into

Table 1. Mechanisms responsible for generating diversity of steroid hormone responses

Mechanism of diversity	Component of receptor action
Agonist, antagonist	Ligand
Receptor gene expression Activating or inactivating enzymes Binding proteins (extra- or intracellular)	Target tissue
Cytoplasmic versus nuclear Isoforms-differential Splicing Gene duplication	Receptor
Hetero- or homodimers DNA binding factors	Dimers
Antagonist isoforms Squelching	Nuclear factors
Consensus versus nonconsensus Number of copies Position Proximity of other response elements	DNA response elements
Gene-specific factors Cell-specific factors	Transactivation

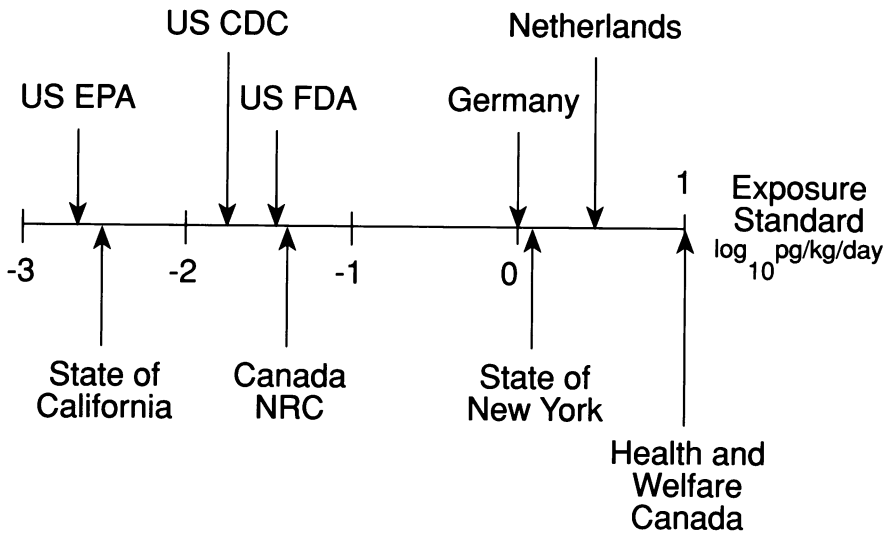


Figure 4. Range of risk assessments made by various U.S. regulatory agencies and other countries.

clonally expanded progeny. Promotion is the enhancement, via modification in cell growth kinetics, of the initiated cell population by endogenous and/or exogenous factors. Progres-sion refers to additional mutations and/or growth of the tumor to a clinical end stage. Tumor promotion in experimental animals is an operationally established paradigm that has been demonstrated in many tissues. Dioxin is a promoter in the skin and liver of experimental animals (*18–20, 40*).

The general approach of the U.S. EPA for regulation of carcinogens is to use the Armitage–Doll model of carcinogenesis. In this model, the movement of cells from one stage to the other is assumed to be due to a sequence of mutations similar to the step of initiation/fixation discussed earlier. As with any mathematical model, specific forms must be chosen for the rate constants that define the process. The EPA formulation of this model assumes the mutation rates are a linear function of dose and are constant over time. EPA generally uses a 95% upper-confidence limit of this formulation of the multistage model for cancer risk assessment (*41*). This model, using the 95% upper-confidence limit on the linear term is referred to as the linearized multistage model (LMS). In addition to chemical carcinogens that increase mutation rate, the linearized mathematical properties of the multistage model can be appropriate for a larger class of mechanisms. In particular, if a compound's action is additive to background biological processes, then a linear response is predicted at low doses under rather general conditions (*42,43*). Therefore, for practical modeling purposes, it is important to address whether biological knowledge and data on carcinogen action can fit the general dose–response shape predicted by the LMS.

For other toxicological endpoints such as terata, target organ toxicity, and acute toxicity, a different approach has been used. For these endpoints, safety factors or uncertainty factors have been used to estimate no-effect exposure levels. This approach is used by the World Health Organization to set acceptable daily intakes for direct and indirect food additives. In contrast, EPA policy assumes the dose–response curve for excess carcinogenic risk to be linear through dose zero. As discussed previously, several mechanisms could generally lead to this form of response, including direct mutational activity of the chemical agent and/or additivity to background rate of tumor formation. Because TCDD does not bind covalently to DNA and appears to exert its effects through receptor action, risk assessments for TCDD must be carefully reexamined. Countries or agencies that use the LMS have much higher estimates of risks than countries that use the safety factor approach (Fig. 4). For example, EPA, using the LMS, estimates that a chronic exposure of 6 fg TCDD/kg day causes an increased risk of 1 cancer in 1 million people exposed to that level. In contrast, Health and Welfare Canada has established a safe exposure level of 10 pg/kg/day using a safety factor approach (*8*). Clearly, Canadians are not 1600 times more sensitive to any adverse health effects of dioxin than Americans, so these differences in risk estimates clearly underscore the huge amount of uncertainty created by selection of various risk assessment methodologies. Although more mechanistically based risk assessments will still contain significant uncertainty, we believe that they constitute an improvement over existing default approaches. As previously stated by Greenlee and collaborators (*44*):

> Neither the position taken by U.S. EPA or by Environment Canada (and several other countries such as Germany and the Netherlands) is based on any detailed mechanistic understanding of receptor-mediated interactions between dioxin and target tissues. Biologically based strategies use knowledge of the mechanistic events in the various steps in the scheme for risk assessment. Interspecies extrapolation strategies should be conducted based on how these mechanistic steps vary from species to species. There are numerous steps that can be examined mechanistically, and fairly ambitious programs have been proposed to examine the mechanistic details of many or most of these individual steps.

There are a number of issues that must be considered in the use of biologically based models to estimate dioxin's risks. One complicating factor is that exposure data in humans indicate that the general population has a body burden TCDD in the range of 5–10 ppt lipid adjusted (*45*). The relationship of this body burden to enhanced risk of disease, if any, is not known, although it does appear that enzyme induction is likely to occur by this exposure level (*35,36*). In any event, we need to gain a clearer understanding of the molecular/toxicological consequences of background exposure to TCDD and its structural analogs and dose–response relationships for incremental increases in exposure. Two other factors affect our ability to understand and reduce background exposures of the human population. First, it is estimated that the background exposure to TCDD is 0.3 pg/kg/day based on current body burdens, but we do not know all the sources that are responsible for this body burden. This information, coupled with the biological and ecological persistence of TCDD, may make it difficult to significantly reduce current human body burdens of TCDD. Second, TCDD is prototypical for a broad class of chemicals that bind the Ah receptor (*11*), so we need to obtain more accurate estimates of the total exposure to TCDD-like compounds and of the biological potency of those compounds. Although dose–response relationships for enzyme induction appear to be similar between rodents and humans (*46,47*), TCDD-mediated human disease may exhibit dose–response relationships different from enzyme induction. In

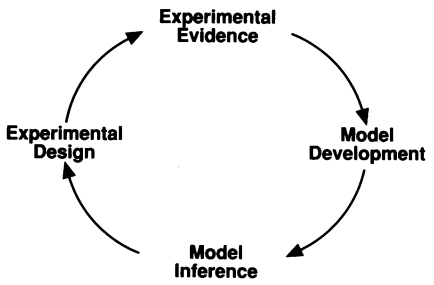


Figure 5. Developing a mechanistically based mathematical model.

addition, the role of natural ligands has to be addressed.

The current effort by the EPA to reevaluate the risk of exposure to dioxins is being called a "biological basis for risk assessment." The underlying premise is that dioxins may be a special case for nonmutagenic, receptor-mediated carcinogens. The goal of this reassessment is to consider more mechanistically based models that are sufficiently credible to the scientific community.

As discussed earlier, several important factors have been generally accepted. First, TCDD is a member of a class of xenobiotics (and probably natural products) that is nonmutagenic, binds to a cellular receptor, and alters cell growth and development. Second, a significant amount of information is available for developing approaches to estimate risks from exposure to this compound, and the default position of directly applying the LMS as a function of dose needs to be reevaluated. Third, the biology of receptor-mediated events should be included in any modeling for TCDD. The goal of mechanistic modeling should be to use as much data as possible to reduce these uncertainties and to identify the areas where data gaps exist.

One difficulty with a novel, albeit biologically based, approach is that it is replacing paradigms (safety factors and LMS models) upon which the U.S. government's risk assessments have been based. There is no *a priori* reason to believe that a model based on greater experimental evidence will be more or less conservative than the LMS model. However, basing the modeling on a mechanistic understanding of the biochemistry of TCDD-induced toxicity should increase our confidence in the resulting risk estimates.

Considerations for Modeling for TCDD

Because there appears to be a strong scientific justification for reevaluating dioxin's risks, we believe that it is appropriate to review strategies and approaches for developing biologically based dose–response models for dioxin. These models provide the scientific foundation for the reevaluation. Mathematical modeling can be a powerful tool for understanding and combining information on complex biological phenomena. The development and use of mechanistically based mathematical models is best described by cycles which are illustrated by Figure 5. The beginning point is generally a series of experiments studying a xenobiotic agent. The experimental results (data) can indicate a mechanism, which leads to the creation of a mathematical model. The model is used to make inferences, which are then validated against the existing knowledge of the effect and the xenobiotic agent being studied. This process can then lead to new experiments and further laps through this model development loop. On each pass through the loop, the model either gains additional validation by predicting the new experimental results, or it is modified to encompass new results without sacrificing its base in previous results. In either case, subsequent loops through the model generally increase our confidence in accepting (or rejecting) a final model. However, it may be difficult or impossible to quantify this confidence using a statistical measure.

Confidence in any one model not only depends on the information available for that compound, but it is also supported by the information available on other systems that act similarly and for which models have already been developed. In the case of TCDD, the modeling effort is greatly enhanced by existing information on the receptor-based system, general work in physiologically based pharmacokinetic models, and tumor incidence modeling.

There is no one model development loop for any given compound or effect. Instead, there are numerous pathways leading to the development of a mechanistic model. In most comprehensive modeling, there are many smaller model development "circuits" that make up the larger, overall model. For example, a mechanistic approach to TCDD-induced carcinogenicity must include models of exposure, tissue distribution, tissue diffusion, cellular biochemistry, cellular action, tumor incidence, and cancer mortality. At each stage and for each model, data must be collected and understood for the model to be valid and acceptable as a tool for understanding the observed effects and for predicting the effects of TCDD outside of the relatively limited range of experimental findings.

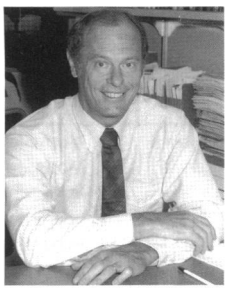
The use of mechanistically based modeling to extrapolate risks of exposure patterns and doses outside the range of the data is in its infancy. Even though there may be much confidence in the ability of the model to predict experimental results, especially for biochemical events, there could be little confidence in the ability of the model to predict outside the range of data for more coordinated biological responses such as cancer. The use of models in risk assessment thus demands careful scrutiny of the behavior of the model under a variety of exposure scenarios. This scrutiny has not generally been applied in science to the use of mechanistic models. It is important to note that mechanistic modeling has a role to aid in explaining and understanding experimental results, separate from its use in risk assessment. Our confidence in the methods used in mechanistic modeling will differ with use.

Table 2. Examples of levels of information available for estimating parameters in dose–response modeling.

Level	Parameter
Organism	Morbidity
	Mortality
	Fertility
	Improper development function
Tissue	Hyperplasia
	Hypertrophy
	Tumorigenesis
	Chemical distribution disposition
Cell	Mitosis
	Cell death
	Cytoarchitectural pathology
Biochemical	Gene expression
	Protein levels
	Receptor binding
	Adduct formation

In any modeling exercise, the major components of the model revolve around the estimation of model parameters using statistical tools. These tools range from simple techniques, such as estimating a mean, to complicated approaches, such as estimation via maximizing a statistical likelihood. Estimating parameters is tied to the data available to characterize the model. The way in which data are used to obtain estimates of the model parameters is the major component in determining the confidence placed in any mathematical model.

In modeling biological phenomena, the data can be divided into four broad categories, as shown in Table 2. At the top are effects on the whole animal. Examples of data included in this category include survival of the organism, its ability to reproduce, and its ability to properly function. The levels of data then decrease and become more specific, going from whole organism to tisssue/organ system responses to cellular responses, and finally down to biochemical responses in the cell. The data in Table 2 range from very general (often no more detail than mortality data) to highly specific mechanistic data dealing with the interactions between molecules.



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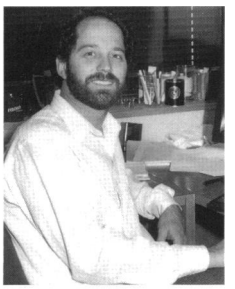

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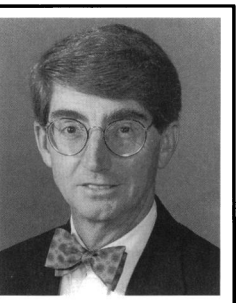

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All of this information is relevant and must be incorporated into a mathematical model aimed at understanding the specific biological response.

The goal of mechanistic models generally applicable to risk asssessment is to predict outcomes, whether at the organism level (e.g., survival) or at the tissue level (e.g., carcinogenicity). In building such a model, it is the goal of mechanistic modeling to explain most, if not all, of the available data. Mathematical models that incorporate parameters which are mechanistic in nature do not automatically constitute "mechanistic models." The types of data available for the model and the method by which these data are incorporated into the model determines if a model is truly "mechanistic": one that is soundly based on the biology rather than simply fitting a curve to the same data.

There are two basic ways biological effects can be estimated. The first and most common approach is a "top-down" approach. In the top-down approach, data on the effect of interest (e.g., carcinogenicity) is modeled directly by applying statistical tools to link the observed data (e.g., tumor incidence data from a carcinogenicity experiment) to a model (e.g., the multistage model of carcinogenesis). This approach is extremely powerful in its ability to describe the observed results and to generate hypotheses about model parameters and the potential effects of changes in these parameters. Where this modeling approach begins to lack credibility is in its ability to predict responses outside the range of the data currently being evaluated. Even when the model being applied to the data is mechanistic in the sense that the model parameters are tied to some mechanism for the toxic effect (e.g., mutation rates and molecular effects), without direct evidence concerning the value for this parameter or even evidence supporting the particular structure of the model, one is basically left with a curve fit to the data. The historical application of the LMS in risk assessment for carcinogens has been used in this fashion.

True mechanistic modeling must be viewed differently. In this case, the model structure and the parameters in the model are derived in a "bottom-up" fashion. The mechanistic parameters in the model are estimated directly from mechanistic data rather than from effects data or data one level higher in the hierarchy of data illustrated in Table 2. The goal of true mechanistic modeling is to explain all or most known results relating to the process under study in a way that is biologically reasonable and soundly rooted in the data at hand. In this case, biological confidence in predictions from the model would be much higher than that from the curve-fitting approach. The major difference between the application of a "curve-fit" model in basic biology and that in risk estimation is that in basic biology one is creating hypotheses which at some point can be tested. In risk estimation, it is unlikely that one will ever be able to validate extrapolated risk estimates.

In practice, it is difficult to completely eliminate curve fitting from mechanistic modeling. At some point in the modeling process, gaps must be filled relating the modeled mechanistic effects to the observed toxic effects. It is generally at this point that some amount of curve fitting is necessary to calibrate the mechanistic response to the toxic effect. Although not technically mechanistic modeling, this combined approach is preferred to simple curve fitting when inferences outside of the range of the toxic effects data are to be made.

Mechanistic modeling does not necessarily provide a precise estimate of risk of a toxic effect outside the range of the data or even necessarily more precise or even im-proved estimates of risk than curve fitting alone. Without data (as in the case of extrapolation), the statistical issue of the accuracy of a prediction cannot be easily addressed. Thus, although there may be a greater deal of biological confidence in extrapolated results, it is unlikely that an increased statistical confidence can be demonstrated. However, for each level and type of data, there are ranges of exposure beyond which it is impossible to dem-onstrate an effect given the practical constraints on the experimental protocols. In general, effects can be demonstrated at lower exposures for more specific data compared to the more general data (Table 2). When this is the case, there may be both increased biological confidence in extrapolated results and increased statistical accuracy. This does not imply that models derived through curved fitting should always be given less weight.

Many additional issues are related to the frequency of the use of the model development loop in trying to understand a biological mechanism. One issue of considerable importance is experimental design. For mechanistic modeling aimed at risk assessment, we are just beginning to understand the types of experiments that may benefit the risk estimation process.

Thus, now is the perfect time to consider the types of designs best suited to addressing problems specific to risk assessment. In most experimental designs one would have a mechanism in mind, qualitatively describe that mechanism, form the structure of a mechanistic model, make educated guesses about the parameters of this model, and then use the quantitative model to develop experimental designs that are optimal at characterizing the mechanism.

For exploring and examining the ability of mechanistic modeling to improve the accuracy of quantitative risk assessment, TCDD can be considered as a prototype. The database for a mechanistic modeling approach to TCDD is extensive and contains a considerable amount of information on low-dose behavior. In addition, there is reasonably good concordance between human data and experimental evidence in animals. On the other hand, some aspects of the mechanism by which TCDD induces its effects have not been modeled extensively, and thus we are in the early loops through the model development cycle shown in Figure 5. As a result, several competing mechanistic theories may agree with the existing data, adding to the uncertainty in any projected risk estimates. This outcome is inevitable for a novel mechanism and for the application of the technology of mechanistic modeling to a new area. As stated earlier, mechanistic modeling has a role to aid in explaining and understanding experimental results, beyond its proposed use in risk assessment. Our confidence in the methods used in mechanistic modeling will differ depending on the history of its use. As we know more about the limitations of current data and current methods for the application of mechanistic models to risk estimation, we can improve experimental designs and significantly improve the process.

Dioxin is an example of early attempts to develop mechanistic models for risk assessment. Scientists have gained considerable insights into the complex network of events that affect the mechanisms whereby receptor-mediated events regulate gene expression and mitotic activity, and the scientific foundation, although far from complete, is sufficient to warrant the development of biologically based dose–response models for the effects of dioxin. However, we must be cautious in judging the overall and immediate utility of mechanistic modeling in risk assessment based on our experiences with dioxin.

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